



## Research report

## An NK<sub>1</sub> receptor antagonist affects the circadian regulation of locomotor activity in golden hamsters

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### Abstract

Substance P (SP) is a neuromodulator which may participate in the photic regulation of the circadian timing system in mammals. The biological effects of SP are mediated by interaction with specific receptors, designated as NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub>. The NK<sub>1</sub> subtype receptor is expressed in the circadian system. Experiment 1 was designed to test whether an NK<sub>1</sub> antagonist mimics the effects of dark pulses. Hamsters were housed in constant lighting conditions, either constant darkness or constant light (around 250 lx), and they received an i.p. injection of either the specific NK<sub>1</sub> receptor antagonist, L-760,735 (5 mg/kg), or saline during the mid-subjective day, a time when dark pulses cause a phase-advance in circadian rhythm of locomotor activity. After treatment with the NK<sub>1</sub> antagonist, significant phase-advances of wheel-running activity rhythm were found in constant light, but not in constant darkness. Experiment 2 was designed to test the ability of the NK<sub>1</sub> antagonist to block the phase-delaying and/or the phase-advancing effects of light in animals kept in constant darkness. Phase-advances of locomotor activity rhythm that can normally be induced by light pulses given during the late subjective night were markedly reduced by pre-treatment with the NK<sub>1</sub> antagonist. By contrast, phase-delays that can be induced by light pulses given during the early subjective night were unaffected by the NK<sub>1</sub> antagonist. These data support the hypothesis that SP within the circadian system may, by interacting with NK<sub>1</sub> receptors, modulate photic responses of the SCN pacemaker. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Circadian rhythm; Suprachiasmatic nucleus; Intergeniculate leaflet; Phase resetting; Tachykinin; NK<sub>1</sub> receptor

### 1. Introduction

In mammals, one of the main components of the circadian timing system is the retinohypothalamic tract (RHT), which conveys photic cues from the retina to the suprachiasmatic nucleus (SCN), site of the primary circadian pacemaker. Photic information is the most potent synchronizer of the SCN pacemaker and the integrity of the RHT is critical for the photic synchronization (entrainment) of the circadian rhythms regulated by the SCN [15,21]. Within the RHT, glutamate is considered to be the main neurotransmitter mediating photic inputs to the SCN [8]. Another component of the circadian timing system, the intergeniculate leaflet (IGL), receives direct retinal inputs and, in turn, projects to the SCN through the geniculohypothalamic tract (GHT). The GHT contains neuropeptide Y (NPY), enkephalin, and also  $\gamma$ -aminobutyric acid (GABA) [21]. The IGL via the GHT may modulate the entraining effects of light on the SCN [7,11]. In addition to light, a wide variety of factors can phase-shift the free-running circadian pacemaker in hamsters. According to the pattern of the phase-response curves (PRCs), these factors are classified in two families of PRCs [36]: (1) those PRCs for photic stimulation in constant dark [6,38], and (2) those PRCs for either dark pulses in constant light [3,9] or non-photoc stimuli in constant lighting conditions (e.g., [32,41]).

The tachykinin substance P (SP) is a neuromodulator that may participate in the regulation of the circadian timing system. SP-immunoreactive cells and fibers have been reported in the SCN of rodents [12,18,39]. SP has also been observed in some ganglion cells in the mammalian retina [4] and pre-protachykinin-A mRNA encoding

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for SP is expressed in the SCN of rats [18]. The biological actions of tachykinins, including SP, are mediated through specific cell surface receptors, of which three subtypes ( $NK_1$ ,  $NK_2$ , and  $NK_3$ ) have been identified. Expression of mRNA for  $NK_1$  subtype receptor and immunoreactive binding sites are found in the retina [5,16] and within the laterodorsal region of the SCN [17,39]. The RHT may contain SP because enucleation can reduce the density of SP-immunoreactive fibers in the SCN of rats [18,39], although other studies using both rats and hamsters have failed to detect this change [12,23,37]. Because contacts between degenerating retinal fibers (after enucleation) and SP-immunoreactive dendrites were found in the SCN [39], SP may influence the photic entrainment at that level. Furthermore, there is a dense plexus of SP-immunoreactive fibers in the IGL [19] that is unaffected after enucleation [12]. Because mRNA encoding for SP and  $NK_1$  subtype receptor are expressed in the IGL [17], SP innervation of the IGL may be involved in the regulation of circadian rhythmicity.

In vivo injections of Spantide, a non-specific SP receptor antagonist, reduce the light-induced expression of Fos in the SCN [1]. Injections of SP in hamsters kept in constant dim-red light induce small phase-delays during the early subjective night but have no phase-shifting effects during late subjective night [25]. SP can stimulate SCN cells in vitro and it also potentiates their glutamate-induced activation [24,35], probably via  $NK_1$  receptors [35]. Moreover, the phase-shifting effects of SP on the firing activity rhythm of SCN neurons in vitro are similar to those of light pulses on circadian phase in animals housed in constant darkness [34]. Taken together, these anatomical and physiological data suggest that SP is involved in the photic entrainment of the circadian timing system.

The  $NK_1$  receptors found in the SCN, IGL and retina [5,16,17,39] may mediate the effects of SP on the circadian system. In this context, we hypothesized that injections of an  $NK_1$  antagonist may (1) mimic the effects of dark pulses in animals kept in constant light and (2) prevent the phase-shifting effects of light in animals housed in constant dark. In Experiment 1, hamsters were kept in constant lighting conditions, either in complete darkness or in constant light. If an  $NK_1$  antagonist can mimic a dark pulse, then it should have no effect in animals free-running in constant darkness. An injection of  $NK_1$  antagonist in constant light, on the other hand, may phase-shift the circadian rhythm of locomotor activity. Experiment 2 was designed to test the ability of an  $NK_1$  antagonist to block the phase-delaying and/or the phase-advancing effects of light pulses in animals kept in constant darkness.

## 2. Materials and methods

Male golden hamsters (*Mesocricetus auratus* Lak:LVG/SYR) (10–14 weeks old) were purchased from

Charles Rivers (Newfield, NJ). Animals were maintained in a temperature-controlled room with a light–dark (LD) cycle consisting of 14 h of light and 10 h of darkness (i.e., LD 14:10). During daytime, light intensity was about 300 lx at the level of the cages. Food (Harlan Teklad laboratory chow, Madison, WI) and water were available ad libitum. A fan provided constant fresh air flow as well as masking noise. After at least 2 weeks to the exposure to the LD cycle, animals were transferred to either constant lighting conditions or constant darkness depending on the experiment. Experimental procedures and animal maintenance under constant darkness were performed with the aid of an infrared viewer (Find-R-Scope, FJW Optical system, Palatine, IL). Hamsters were housed individually in cages equipped with a running wheel (diameter: 17 cm) that activated a microswitch on each revolution. Wheel-running activity was recorded using the Chronobiology Kit (Stanford Software Systems, Stanford, CA).

Experiment 1 was designed to test the hypothesis that injections of an  $NK_1$  antagonist can mimic the effects of dark pulses in animals kept in constant light. Two groups of 6 hamsters each were kept in constant darkness and constant light (range: 150 to 400 lx), respectively. After a minimum of 10 days, half the animals in each group received a single i.p. injection of 5.0 mg/kg of the selective  $NK_1$  antagonist, L-760,735 [2-(R)-(1-(R)-3,5-bis(trifluoromethyl)phenyl)ethoxy)-4-(5-(dimethylamino-methyl)-1,2,3-triazol-4-yl)methyl-3-(5-phenyl)morpholine] in 0.5 ml of saline. This compound binds selectively and with sub-nanomolar affinity to the  $NK_1$  subtype of tachykinin receptors of humans, guinea pigs and gerbils, and with slightly lower affinity to rat  $NK_1$  receptors (receptor data provided by the laboratory of Dr. M. Cascieri at Merck Research Laboratories). The other half received 0.5 ml of vehicle (saline). Injections were performed at circadian time (CT) 8 (4 h before the time of activity onset, designed as CT12). This dose and CT were chosen according to data obtained in a preliminary experiment where this dose of L-760,735, but not the enantiomer L-781,773, which lacks  $NK_1$  antagonist activity, can phase-shift the free-running rhythm of animals kept in constant light only when injected during the mid-subjective day (Naylor and Turek, unpublished data). The experiment was then repeated after 10 days, using a cross-over design in which the animals received the alternate treatment. The circadian period was assessed by the  $\chi^2$  periodogram (Chronobiology Kit software) over the 10 days before and after the treatment.

Experiment 2 was designed to test the ability of the  $NK_1$  antagonist to block light-induced phase-shifts. Six groups of 10 hamsters were housed in constant darkness. After at least 10 days, half the hamsters ( $n = 30$ ) received a single i.p. injection of 5.0 mg/kg of the  $NK_1$  antagonist L-760,735 in 0.5 ml of saline, while the other half ( $n = 30$ ) received 0.5 ml of saline. Thirty minutes after the treatment, animals were exposed to a light pulse at either

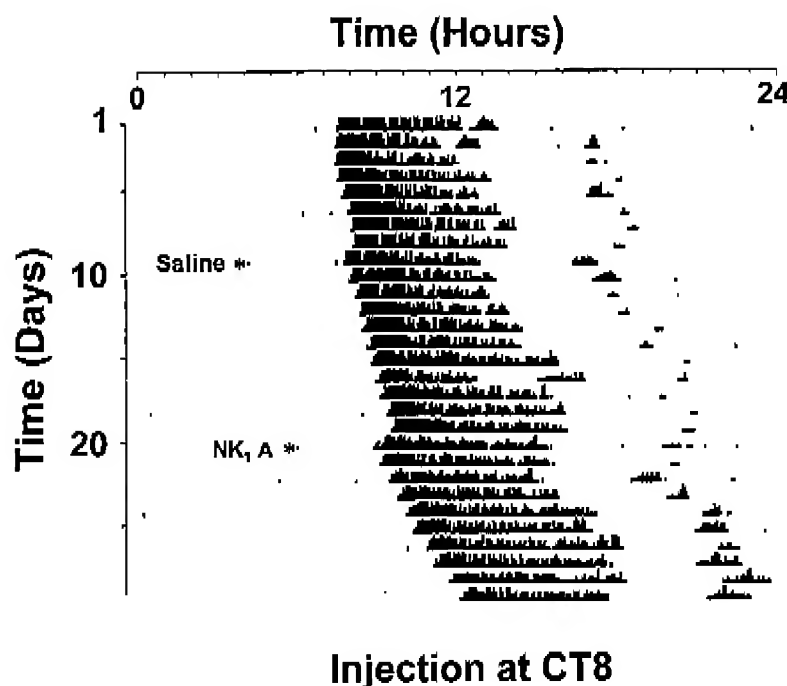


Fig. 1. Wheel-running activity over a 24-h period of a hamster kept in constant white light. On 2 separate occasions, this animal was treated with saline or NK<sub>1</sub> antagonist L-760,735 (NK<sub>1</sub> A; 5 mg/kg i.p.) at CT8. Time of injection is indicated by the asterisk.

CT14, a time when light induces large phase-delays of the circadian rhythm of locomotor activity in hamsters, or CT19, a time when light induces large phase-advances [6,38]. For light stimulation, individuals were transferred from their own cages to a chamber (diameter 11 cm, height 6 cm) inside a photic stimulation device described previously [22]. For a given CT ( $n = 30$ ), each group of 10 animals received one of 3 possible light pulses: 20 lx of white light for 10 min, 40 lx of white light for 15 min, or 60 lx of white light for 15 min. Light intensity was determined using a digital photometer (Sper Scientific). The characteristics of these white light pulses were chosen according to the magnitude of the circadian phase-shifts they induced in other studies [29,30]. After the light pulse, all hamsters were returned to their home cages. Ten days later, the same photic procedure was repeated for all hamsters with individuals then receiving the alternate injection of the NK<sub>1</sub> antagonist or saline. The dose of 5.0 mg/kg of L-760,735 was chosen according to data obtained in a preliminary experiment where 0.5, 1.5, or 5.0 mg/kg of L-760,735 were injected 30 min prior to a light pulse started at CT19. Only the highest dose (i.e., 5.0 mg/kg) of the NK<sub>1</sub> antagonist L-760,735 was effective in limiting the light-induced phase-advance. Injection of 5.0 mg/kg of the enantiomer L-781,773, which lacks NK<sub>1</sub> antagonist activity, had no effect on the light-induced phase-advance (Challet and Turek, unpublished data).

Experiment 3 was designed to test whether peripheral administration of an NK<sub>1</sub> antagonist that does not cross the blood-brain barrier, such as L-743,310 [40], is effective in blocking the light-induced phase-advances of the circadian

rhythm of locomotor activity. A group of 10 hamsters were housed in constant darkness. After at least 10 days, half the hamsters ( $n = 5$ ) received a single i.p. injection of 5.0 mg/kg of the NK<sub>1</sub> antagonist, L-743,310 {5-(3,5-bis(trifluoromethyl)phenyl)-1-(3-indolyl)-2-(3-(4-(1-methyl)quinuclidinyl)ureido)-3-pentanone} in 0.5 ml of saline, while

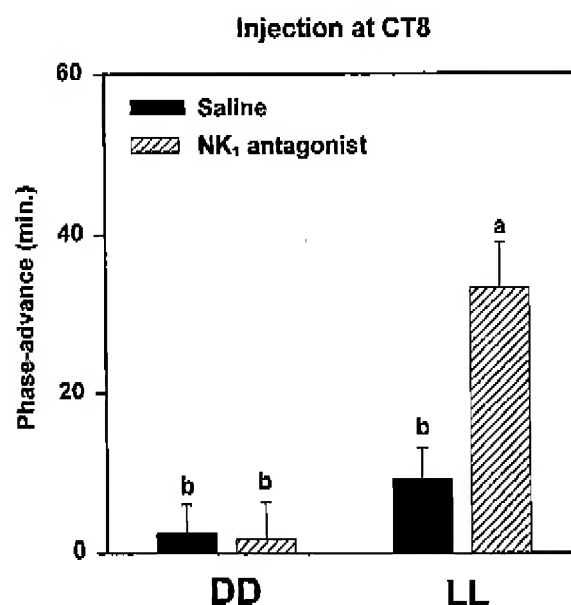


Fig. 2. Phase-advances of hamsters kept in constant darkness or constant white light. On 2 separate occasions, each animal was treated with saline or NK<sub>1</sub> antagonist L-760,735 (5 mg/kg i.p.) at CT8. Means  $\pm$  S.E.M. Groups with different letters are significantly different from one another ( $P < 0.05$ ).

the other half ( $n=5$ ) received 0.5 ml of saline 30 min before a white light pulse (20 lx of white for 10 min) started at CT19. The photic stimulation device was similar to that described in Experiment 2. Ten days later, the same photic procedure was repeated for all hamsters with individuals then receiving the alternate injection of the NK<sub>1</sub> antagonist L-743,310 or saline.

To determine the phase-shifts of the circadian rhythm of locomotor activity, regression curves were eye-fitted to the onsets of locomotor activity for the last 8–10 days before the treatment and projected to the day of the treatment. Similarly, regression lines fitted to the onsets of activity during the 8–10 days after the treatment were retroprojected to the day of treatment. The magnitude of the

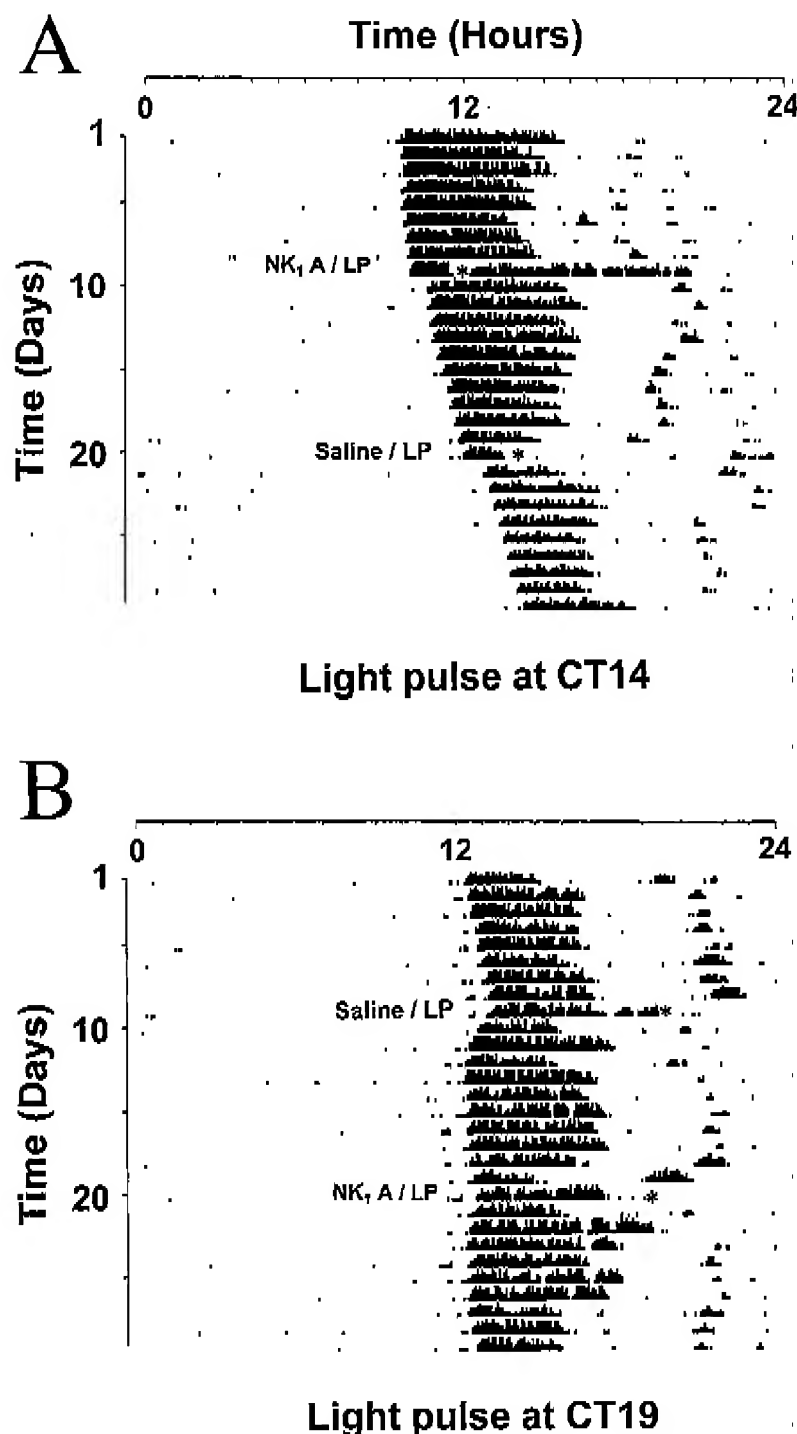


Fig. 3. Wheel-running activity over a 24-h period of 2 hamsters kept in darkness. On 2 separate occasions, these animals were treated with saline or NK<sub>1</sub> antagonist L-760,735 (NK<sub>1</sub> A; 5 mg/kg i.p.) 30 min before a white light pulse (LP; 60 lx for 15 min) started at CT14 (A: upper panel) and at CT19 (B: lower panel). Time of injection is indicated by the asterisk.

phase-shift was calculated as the difference between these two lines.

Values are means  $\pm$  S.E.M. Two-way analysis of variance with repeated measures followed by a Student-Newman-Keuls test were performed using SigmaStat software (Jandel Scientific, San Rafael, CA). Because there was no effect of order of treatment ( $P > 0.05$ ), the corresponding data from animals receiving the same injection (NK<sub>1</sub> antagonist or saline), but the reversed order of treatment, were combined together for the final analysis.

### 3. Results

In Experiment 1, the main effects of both the light intensity (i.e., constant darkness vs. constant light) and the treatment (i.e., injection of the NK<sub>1</sub> antagonist L-760,735 vs. saline at CT8) were significant ( $F(1,10) = 22.1$ ,  $P < 0.001$ , and  $F(1,10) = 5.4$ ,  $P < 0.05$ , respectively). In addition, the interaction term was significant ( $F(1,10) = 6.2$ ,  $P < 0.05$ ), indicating that the effect of the NK<sub>1</sub> antagonist depended on which background light intensity was present. Injections of the NK<sub>1</sub> antagonist L-760,735 at CT8 led to significantly larger phase-advances than those induced by saline injections in constant light ( $33 \pm 6$  vs.  $9 \pm 4$  min, respectively), but not in constant darkness ( $2 \pm 5$  vs.  $3 \pm 3$  min, respectively; Figs. 1 and 2). There were no significant changes in the circadian period before and after the treatment ( $F(1,5) = 0.01$ ,  $P > 0.1$ ) in animals kept in constant darkness and receiving an injection of the NK<sub>1</sub> antagonist or saline. By contrast, regardless of the treatment (NK<sub>1</sub> antagonist L-760,735 or saline), the circadian period of animals kept in constant light was lower before compared to after the treatment ( $24.16 \pm 0.06$  vs.  $24.37 \pm 0.04$  h, respectively;  $F(1,5) = 42.2$ ,  $P < 0.01$ ), an effect probably due to tonic effects of constant light upon the circadian clock.

In Experiment 2, a light pulse applied at CT14 resulted in phase-delays of free-running locomotor activity (Figs. 3 and 4). The phase-delays were larger when the intensity of the light pulses increased ( $F(2,27) = 11.5$ ,  $P < 0.001$ ). There was no overall effect of the treatment (i.e., injection of NK<sub>1</sub> antagonist vs. saline at CT13.5;  $P > 0.05$ ), and no effect of the treatment  $\times$  intensity of light pulse interaction ( $P > 0.05$ ). This demonstrates that the NK<sub>1</sub> antagonist L-760,735 had no effect on the light-induced phase-delays, regardless of the intensity of the light pulses (Fig. 4).

A light pulse applied at CT19 resulted in phase-advances of circadian rhythm of wheel-running activity (Figs. 3 and 4). The higher intensity of the light pulses induced larger phase-advances ( $F(2,27) = 5.9$ ,  $P < 0.01$ ). The effect of treatment (i.e., injection of NK<sub>1</sub> antagonist L-760,735 vs. saline at CT18.5) was highly significant ( $F(1,27) = 38.2$ ,  $P < 0.0001$ ), indicating that the NK<sub>1</sub> antagonist, L-760,735, reduced the magnitude of photic phase-advances. The treatment  $\times$  intensity of light pulse

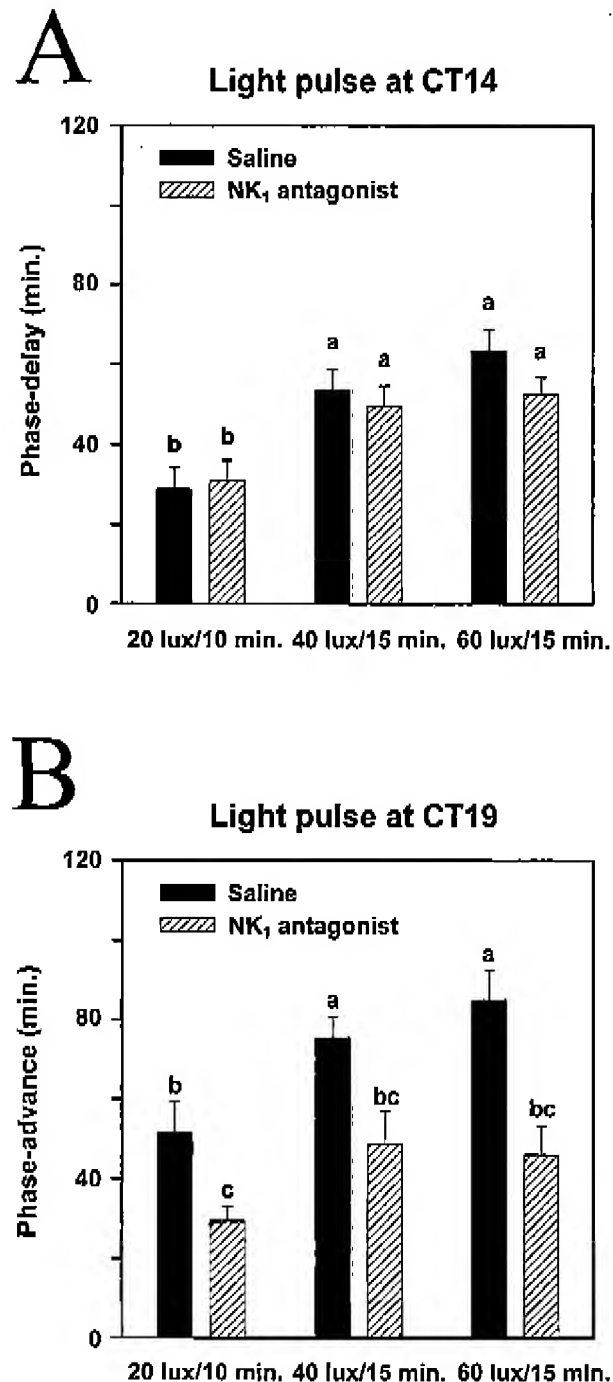


Fig. 4. (A) Phase-delays of hamsters in response to different white light pulses at CT14. (B) Phase-advances of hamsters in response to different white light pulses at CT19. The same photic stimulus was given to same animals on separate occasions 30 min after they were treated with saline or NK<sub>1</sub> antagonist L-760,735 (5 mg/kg i.p.). Means  $\pm$  S.E.M. Groups with no letters in common are significantly different from one another ( $P < 0.05$ ).

interaction was not significant ( $P > 0.05$ ). The NK<sub>1</sub> antagonist L-760,735 inhibited the light-induced phase-advances regardless of the intensity of the light pulses (Fig. 4).

In Experiment 3, the phase-advance induced by a light pulse applied at CT19 was not significantly different in

animals receiving either L-743,310 (i.e., the non-brain penetrant NK<sub>1</sub> antagonist) or saline at CT18.5 ( $72 \pm 6$  vs.  $73 \pm 7$  min, respectively; paired *t*-test,  $P > 0.05$ ; data not shown).

#### 4. Discussion

The findings reported here indicate that (1) an i.p. injection of a SP NK<sub>1</sub> receptor antagonist (i.e., L-760,735) at CT8 can phase-advance the circadian rhythm of locomotor activity in hamsters housed in constant light; (2) an i.p. injection of this NK<sub>1</sub> antagonist strongly attenuates the light-induced phase-advances at CT19, but has no effect on the light-induced phase-delays at CT14. These data support the hypothesis that SP innervation within the circadian timing system may modulate photic responses of the SCN pacemaker. Given that L-760,735 can cross the blood-brain barrier [33], it is most likely that these specific effects of the NK<sub>1</sub> antagonist on the circadian rhythmicity are due to direct effects in the brain. Indeed, the peripheral administration of the NK<sub>1</sub> antagonist, L-743,310, that does not cross the blood-brain barrier [40], does not block the phase-advancing effects of a light pulse.

Bouts of hyperactivity during the inactive period (e.g., those associated with an injection of Triazolam) are able to feedback to the SCN to induce phase-shifts in the circadian clock [32,42]. The absence of wheel-running activity following NK<sub>1</sub> antagonist injection during the subjective day (Fig. 1) rules out the possibility that the observed phase-changes are due to increased locomotor activity. The phase-advances induced by injections of the NK<sub>1</sub> antagonist injected at CT8 in hamsters housed in constant light, but not in constant darkness, show that the NK<sub>1</sub> antagonist does not act as a classical non-photic stimulus (i.e., one factor that would also phase-shift the circadian pacemaker in constant darkness) [32,41]. Instead, these observations support the hypothesis that the NK<sub>1</sub> antagonist mimics the effects of a dark pulse. The presence of SP-immunoreactive fibers within the RHT [18,39] is equivocal in rodents [12,23,37]. A small cluster of SP-immunoreactive cells, however, is located in the central core of the SCN of hamsters and rats [12,18,39] and a few neurons expressing mRNA for the NK<sub>1</sub> receptor are distributed at the laterodorsal margins of the SCN [17]. A possible inhibition of these receptors may therefore be involved in the phase-shifting effect of the SP antagonist. This hypothesis is further supported by the *in vitro* data showing activation of SCN cells by local application of SP [24,35], and inhibition by an NK<sub>1</sub> receptor antagonist [35]. Previous studies have shown that the SCN pacemaker *in vitro* can be shifted during the subjective day by application of a cAMP analog [26]. In addition, pituitary adenylate cyclase-activating peptide (PACAP), which is present in the RHT, also phase-advances the SCN pacemaker *in vitro* during the middle of the subjective day [10]. This effect is blocked by

a specific cAMP antagonist [10]. Interestingly, the timing of SCN sensitivity to cAMP, PACAP, and an NK<sub>1</sub> antagonist is similar. Thus, the NK<sub>1</sub> antagonist may modulate this or another cAMP-dependent pathway in the SCN during the mid-subjective day.

It is also possible that the NK<sub>1</sub> antagonist acts at a site upstream of the SCN. Given that NK<sub>1</sub> receptors are expressed in the retina [5,16], inhibition of SP activity within the retina may gate the photic cues before they reach the SCN or prevent them to reach the SCN, therefore mimicking a dark pulse. Another possibility is that the NK<sub>1</sub> antagonist during the subjective day acts primarily on SP innervation of the IGL, which contains immunoreactive fibers [19] and NK<sub>1</sub> binding sites [17]. NPY is synthesized in the IGL and released by the GHT into the SCN. Likewise, the PRC to NPY is similar to that of dark pulses in that NPY is capable of phase-advancing the SCN pacemaker during the subjective day [13]. However, injections of NPY, but not the NK<sub>1</sub> antagonist, can phase-shift the SCN clock when applied during the subjective day in constant darkness. Nevertheless, lesions of the IGL impair the phase-shifting effects of dark pulses in hamsters housed in constant light [11]. This finding, therefore, raises the possibility that the effects of the NK<sub>1</sub> antagonist injected at CT8 in constant light (present study) involve activation of the GHT that, in turn, would have phase-shifted the SCN pacemaker.

The NK<sub>1</sub> antagonist greatly attenuates phase-advances caused by light pulses at CT19 in hamsters held in constant darkness. By contrast, light-induced phase-delays at CT14 are unaffected by the NK<sub>1</sub> antagonist. Both light-induced phase-delays and advances are associated with release of glutamate by RHT terminals and subsequent activation of both NMDA and non-NMDA receptors [8]. In spite of the fact that NK<sub>1</sub> receptors are found in dorsal and lateral aspects of the SCN and not in the ventral SCN region to which the RHT projects [17], SP has been suggested to modulate glutamate release from RHT terminals. Several *in vitro* results are consistent with this idea. Application of SP induces phase-shifts in the neuronal activity rhythm of SCN slices with a PRC similar to light pulses [34]. SP can enhance the excitatory responses evoked by glutamate of SCN neurons *in vitro*, probably via NK<sub>1</sub> receptors [35]. Moreover, SP may facilitate release of glutamate from SCN slices (Shibata, unpublished data; cited in Ref. [14]). Thus, *in vivo* injections of the NK<sub>1</sub> antagonist may have reduced the glutamatergic phase-shifting effects at CT19, possibly through an inhibition of glutamate release from RHT terminals. Besides direct effects of SP at the SCN level, i.p. injection of the NK<sub>1</sub> antagonist may also inhibit the SP pathway in the IGL. Application of NPY inhibits glutamate-induced phase-advances and delays of firing activity rhythm of SCN slices [2]. Because microinjections of NPY into the SCN can block light-induced phase-advances [43], we speculate that our NK<sub>1</sub> antagonist treatment at CT18.5 may have acti-

vated the NPY release from the GHT, leading to a decreased glutamatergic neurotransmission. On the other hand, NK<sub>1</sub> receptors are also found in the mammalian retina [5,16]. Blockade of these receptors might prevent their activation by photic stimulation, leading to a reduction of the light-induced phase-shifting effects. However, the reason why this would have occurred during the late night, but not during the early night, is unclear. At the present time, it is not known whether the NK<sub>1</sub> antagonist injected i.p. acts at the level of the SCN, IGL and/or retina.

Photic phase-delays and advances are considered to be processed within the SCN, through functionally separate neurochemical mechanisms. Phase-advances during the subjective night may be dependent on a cGMP signalling pathway [27] leading to activation of cGMP-dependent protein kinase, while phase-delays during the subjective night seem to be cGMP-independent processes [44]. Serotonergic projections from the midbrain raphe nuclei can modulate the photic responses to light in the SCN: serotonergic agonists reduce [30] and antagonists potentiate [31] the magnitude of photic phase-shifts. Because both light-induced phase-delays and phase-advances are affected by serotonergic activity, serotonin might not interfere with the NK<sub>1</sub> antagonist that reduces only the photic phase-advances. Diazepam, a benzodiazepine that can potentiate GABA activity, blocks light-induced phase-advances, but not delays [28], as do NPY [43] and the NK<sub>1</sub> antagonist used in this study. GABA may have a key modulatory role in the circadian system [20]. Thus, interactions between GABA, NPY, and SP neurotransmission may be associated with cGMP-dependent mechanisms underlying photic phase-advances of the SCN pacemaker. Furthermore, in response to light pulses at CT13, c-Fos is expressed mainly in the ventrocaudal region of the SCN. Expression of c-Fos after light pulses at CT18 is more widespread within the SCN, including the rostral and dorsal regions [29], where SP receptors are also observed. Activation of those SCN cells may be critical for photic phase-advances. In accordance with our data, pretreatment with Spantide, a non-specific SP antagonist, reduces Fos expression in the rostral, central and dorsocaudal parts of the SCN after a light pulse at CT16 [1]. In addition to this effect, in the early phase-advance region of the PRC to light in hamsters, it would be interesting to know whether NK<sub>1</sub> antagonists have an effect on light-induced Fos expression during the phase-delay region.

By means of i.p. injections of a selective NK<sub>1</sub> receptor antagonist, our results indicate a role of SP as a neuromodulator of the effects of light on the circadian system. An NK<sub>1</sub> antagonist injected during the subjective day can phase-advance the circadian pacemaker of hamsters housed in constant light, therefore mimicking the effects of a dark pulse. Also, treatment with an NK<sub>1</sub> antagonist reduces specifically the light-induced phase-advances in animals kept in constant dark. Further studies are needed to iden-

tify precisely the role of the SP innervation of the SCN, IGL, and retina in the photic regulation of circadian rhythmicity.

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